Performance Characteristics of Four Immunonephelometric Assays for the Quantitative Determination of IgA and IgM in Cerebrospinal Fluid

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Abstract

Measurement of IgA and IgM in cerebrospinal fluid (CSF) can be useful in the diagnosis of multiple sclerosis and other central nervous system disorders. The Dade Behring (Deerfield, IL) N Latex IgA and N Latex IgM tests on the BN II System and Beckman Coulter (Brea, CA) low-concentration IgA and IgM tests on the IMMAGE Immunochemistry System were evaluated for linearity, imprecision, method comparison, and reference interval verification. Both IgA methods were linear from 1.4 to at least 50 mg/L. Both IgM methods were linear from 0.14 to more than 6 mg/L. The total imprecision of the BN II IgA and IgM methods and the IMMAGE IgA method was less than 10%. The imprecision of the IMMAGE IgM method was 10.2% at 0.49 mg/L and less than 5% at higher IgM concentrations. Method comparison studies indicated that IgA and IgM methods on both instruments showed good comparability. Reference interval studies demonstrated that both methods had similar reference intervals that agreed with published values of less than 6 mg/L for IgA and less than 1.3 mg/L for IgM. Methods for quantifying IgA and IgM in CSF on the BN II and IMMAGE nephelometers perform well and give comparable results.

One of the first reports describing the determination of cerebrospinal fluid (CSF) proteins, including albumin, IgG, IgA, and IgM, by immunonephelometry appeared almost a quarter century ago.[1] The simultaneous measurement of albumin and IgG in CSF and serum samples has a routine role in the diagnosis of multiple sclerosis, and the quantitative determination of CSF IgM to show intrathecal production of IgM is an optional test for the diagnosis of multiple sclerosis.[2] There are a number of situations in which CSF IgG measurements alone are not adequate and quantification of CSF IgA or IgM is useful. The quantitative determination of CSF IgA is of little value in the diagnosis of multiple sclerosis; however, strong intrathecal production of IgA may imply a different diagnosis.[3] Intrathecal IgA production is the most sensitive CSF parameter for cerebral adrenoleukodystrophy and is not seen in patients with adrenomyeloneuropathy.[4] An intrathecal IgA response can indicate a bacterial origin with a response rate of nearly 90% in neurotuberculosis and can provide information about a brain abscess.[3] Intrathecal production of IgM can be seen in acute and chronic diseases, including neuroborreliosis, mumps meningitis, and parenchymatous neurosyphilis.[3] Detection of an IgM response alone is not very useful but in combination with other CSF data can contribute to typical patterns seen in neuroborreliosis, mumps meningitis, or non-Hodgkin lymphoma.

It still can be difficult to find automated methods with sufficient sensitivity for accurate determinations of IgA and IgM in CSF.[5] This study was conducted to evaluate the current performance characteristics of immunonephelometric assays for CSF IgA and IgM from 2 vendors.

Materials and Methods

Low-concentration IgA and IgM reagent kits and CSF protein calibrators were used on an IMMAGE Immunochemistry System (Beckman Coulter, Brea, CA) in accordance with the manufacturer's instructions. N Latex IgA and IgM reagents were used on a BN II System (Dade Behring, Deerfield, IL) in accordance with the manufacturer's instructions. The N Latex IgM method is not available for sale in the United States. The reagents from both vendors use particles to enhance assay performance and facilitate measurement of the low concentrations of IgA and IgM normally present in CSF.

Linearity studies were performed using 2 pools of CSF patient samples, one with a high concentration of IgA or IgM and one with a low concentration, and making dilutions of the high pool with the low pool. Multiple surplus CSF samples containing low, medium, and high concentrations of IgA and IgM were pooled for use in the imprecision studies. Each pool was analyzed in duplicate in each of 2 runs performed daily for 5 days for a total of 20 replicates. Different pools were used on the BN II and IMMAGE analyzers, and, therefore, the mean values for each pool are substantially different.

For method comparison studies, CSF samples were selected from those that had been submitted to the clinical laboratory for immunoglobulin and albumin analyses. For the reference interval study, CSF samples that had been submitted to the clinical laboratory for testing to diagnose multiple sclerosis and that met the following criteria of normality were selected: (1) no oligoclonal bands detected by isoelectric focusing and silver staining, (2) normal concentrations of IgG and albumin and a normal IgG/albumin ratio, and (3) normal albumin and IgG indices. For reference interval studies, a total of 82 samples were used with 21 from male subjects ranging in age from 14 to 83 years and 61 from female subjects ranging from 9 to 82 years. The median age of all subjects was 43 years. Samples were stored at –70°C until analysis. Approval of the institutional review board of the University of Utah Health Sciences Center, Salt Lake City, was obtained for all studies in which samples from human subjects were used.

EP Evaluator Release 4 software (David G. Rhoads Associates, Kennett Square, PA) was used for complex imprecision calculations, Deming regression analysis, calculation of r and $S_{y/x}$, linearity assessment, and reference interval estimation. Reference intervals were determined nonparametrically using the Harrell-Davis approach in this software.

Results

The linearity of each method was assessed. The BN II IgA method was linear from 1.4 to 67.4 mg/L with a maximum deviation from a mean recovery of 100% of 7.7%. The BN II IgM method was linear from 0.14 to 9.05 mg/L with a maximum deviation from a mean recovery of 100% of 7.4%. The IMMAGE IgA method was linear from 1.4 to 50.2 mg/L with a maximum deviation from a mean recovery of 100% of 6.3%. The IMMAGE IgM method was linear from 0.30 to 6.73 mg/L with a maximum deviation from a mean recovery of 100% of 9.4%.

The imprecision data for each method are summarized in **Table 1**. The imprecision of the BN II and IMMAGE CSF

Table 1
Summary of Imprecision Study Results

		Coefficient of Variation (%)			
Instrument*/Pool No.	Mean (mg/L)	Within Run	Between Run	Between Day	Total
IgA					
BN II					
1	2.33	5.6	6.0	0.0	8.2
2	8.61	3.5	2.7	0.8	4.5
3	37.85	2.3	2.1	0.0	3.1
IMMAGE					
1A	2.09	4.8	6.6	4.7	9.4
2A	7.00	1.9	4.4	0.9	4.9
3A	24.1	2.1	2.0	3.5	4.6
IgM					
BN II					
1	0.32	4.7	0.0	3.1	5.6
2	2.80	3.0	0.0	0.6	3.0
3	6.91	2.9	1.2	0.0	3.2
IMMAGE					
1A	0.49	8.0	6.3	0.0	10.2
2A	2.54	1.4	1.4	1.3	2.4
3A	5.50	3.7	0.0	2.8	4.7

* For manufacturer information, see the text.

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IgA assays was comparable with total coefficients of variation of less than 10%, even at the lowest concentration of pooled patient material. In contrast, the BN II CSF IgM assay was considerably more precise than the IMMAGE CSF IgM assay at the lowest concentration of control material assessed that was within the reference interval of both assays. At concentrations of control materials that were above the upper limit of the reference interval, the 2 assays had comparable imprecision.

Method comparison studies for CSF IgA and CSF IgM were performed with patient samples **Figure 1**. The IgA

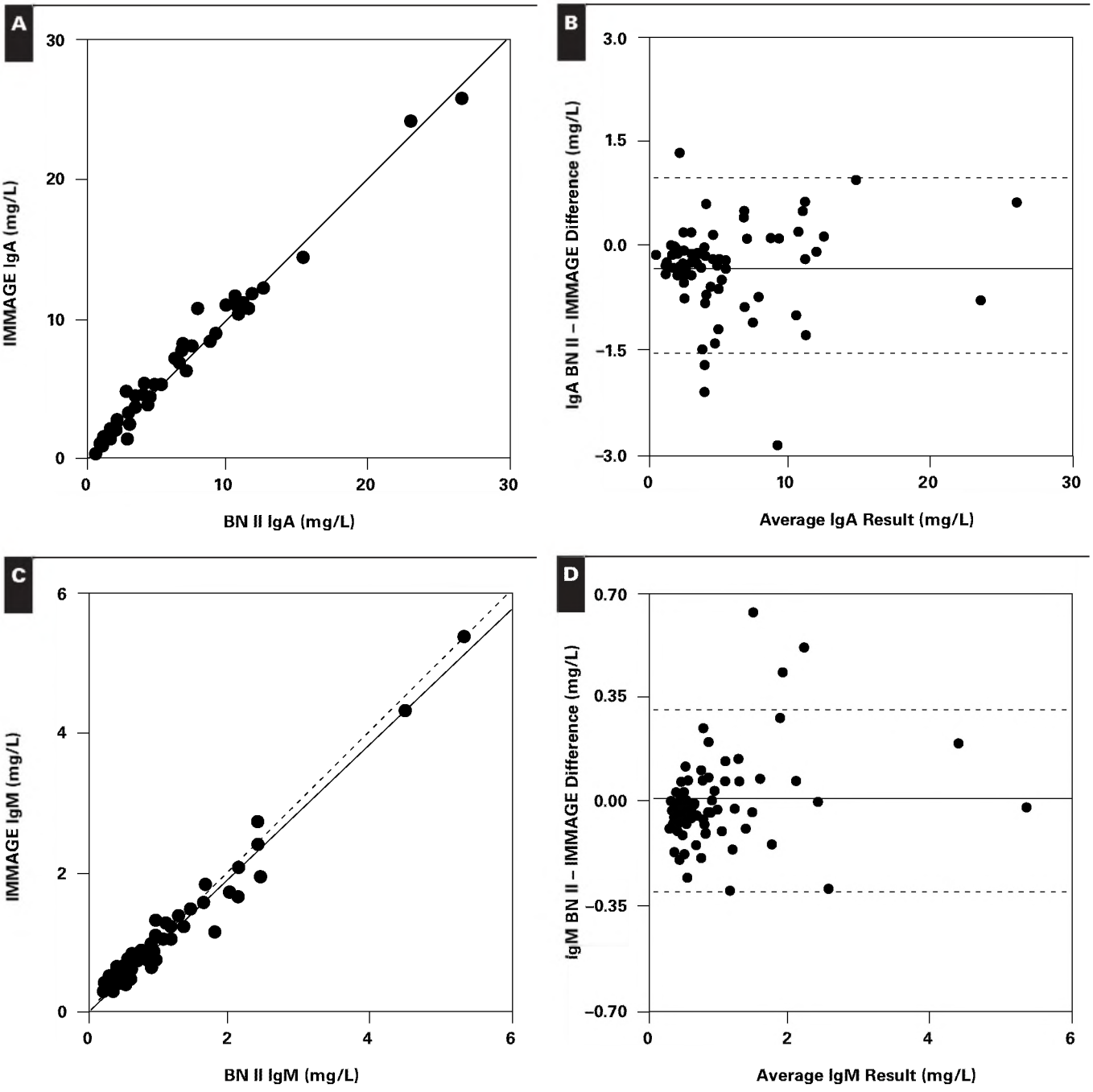

Figure 1 Results of cerebrospinal fluid method comparison studies. **A**, Analysis of 81 samples for IgA by both methods. Deming regression analysis of all data points gave a slope of 0.99 ± 0.03, an intercept of 0.35 ± 0.20, $S_{y/x}$ = 0.62, and r = 0.991. **B**, The mean difference is –0.31 mg/L, and 2 SD is 1.24 mg/L. **C**, Analysis of 77 samples for IgM by both methods. Deming regression analysis of all data points gave a slope of 0.96 ± 0.04, an intercept of 0.03 ± 0.05, $S_{y/x}$ = 0.14, and r = 0.985. **D**, The mean difference is 0.01 mg/L, and 2 SD is 0.30 mg/L. **A** and **C**, Solid lines indicate the Deming regression lines; dashed lines indicate x = y. **B** and **D**, Solid lines indicate the mean differences; dashed lines indicate 2 SD from the means in Bland-Altman analyses. For manufacturer information, see the text.

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methods compared very well by Deming regression with a slope of 0.99 and a correlation coefficient of 0.991. The IgM methods also gave an acceptable comparison with a slope of 0.96 and a correlation coefficient of 0.985. Bland-Altman plots revealed an increased degree of scatter for IgA at concentrations below 10 mg/L and for IgM at concentrations of 2 mg/L.

The nonparametric reference intervals determined in this study were 0.7 to 7.0 and 0.9 to 7.1 mg/L for CSF IgA for the BN II and IMMAGE methods, respectively. Similarly, nonparametric reference intervals were less than 1.2 and less than 1.1 mg/L for CSF IgM determined by the BN II and IMMAGE methods, respectively.

Discussion

Both methods were highly linear across appropriate CSF immunoglobulin concentrations. All methods showed acceptable imprecision except for the IMMAGE IgM, which showed borderline imprecision at CSF IgM concentrations below the upper limit of the reference interval. Method comparison studies demonstrated good comparability for both sets of methods. This indicates a high degree of calibration standardization.

One area that requires further work is the reference intervals. The reference intervals in the package inserts for the 4 assays were all taken from the literature. The reference intervals for the IMMAGE CSF IgA and IgM were less than 2.0 mg/L. The reference cited (Burtis and Ashwood[6]) in the IMMAGE package insert has 2 different reference intervals printed in different sections. On page 517, reference intervals for both IgA and IgM are listed as less than 2 mg/L when measured by radioimmunoassay (RIA).[6] On page 1820, age-specific parametric reference intervals for IgA determined by RIA are listed with an upper limit of 2.3 mg/L, and on page 1821, age-specific parametric reference intervals for IgM by RIA are listed with an upper limit of 0.27 mg/L.[6] The N Latex IgA package insert for CSF lists upper limits of the reference interval of 6 and 5 mg/L based on 2 references.[7,8] Similarly, the N Latex IgM package insert for CSF lists upper limits of the reference interval of 1.3 and 2 mg/L based on the same 2 references.[7,8] A review of the literature revealed 1 additional reference interval study performed for CSF IgM using an enzyme-linked immunosorbent assay method in which the upper reference limit, calculated as the 0.95 fractile, was 0.36 mg/L.[9]

The reference intervals determined in the present study of 0.7 to 7.0 and 0.9 to 7.1 mg/L for CSF IgA for the BN II and IMMAGE methods, respectively, agree best with an upper limit of less than 6 mg/L indicated in the BN II package insert and taken from a single reference.[7] Similarly, the reference intervals of less than 1.2 and less than 1.1 mg/L for CSF IgM determined for the BN II and IMMAGE methods, respectively, agree best with an upper limit of less than 1.3 mg/L indicated in the N Latex IgM package insert and taken from the same reference.[7] The use of CSF-serum quotients for immunoglobulins reduces the influence of individual biologic variability in plasma concentrations on the interpretation of CSF data and may be more useful than the CSF concentrations alone.[10,11] It is noteworthy that the reference intervals for both CSF IgA and IgM are virtually identical for both nephelometers, although the reference intervals published in the package inserts do not reflect this close agreement. Additional studies on appropriate clinical decision thresholds and the use of these assays for the diagnosis and monitoring of multiple sclerosis are warranted.

Conclusions

Methods for quantifying IgA and IgM in CSF on the BN II and IMMAGE nephelometers perform well and give comparable results. These results should facilitate automated measurements of these analytes on a more routine basis. Information about intrathecal production of IgA and IgM can be useful in the diagnosis of a number of central nervous system disorders.

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